



Full Length Article

Promoter Analysis of Cold-responsive (COR) Gene from *Capsella bursa-pastoris* and Expression Character in Response to Low Temperature

Ping Lin¹, Lihua Wu², Donghui Wei², Hu Chen², Mingqi Zhou², Xiaohua Yao^{1*} and Juan Lin^{2*}

¹Research Institute of Subtropical Forestry, Chinese Academy of Forestry, Fuyang, 311400, Zhejiang, People's Republic of China

²State Key Laboratory of Genetic Engineering, Institute of Plant Biology, School of Life Sciences, Fudan University, Shanghai, 200433, People's Republic of China

*For corresponding author: yaohx168@163.com; linjuan@fudan.edu.cn

Abstract

Capsella bursa-pastoris is well adapted to different environments, especially low temperatures, suggesting that it possesses relatively strong tolerance ability to cold. Cold tolerance ability is associated with the accumulation of several cold-induced transcripts. By using a cloning technology, we isolated and sequenced the corresponding COR15 gene from *C. bursa-pastoris*. The putative promoter of CbCOR15 contains *cis-acting* elements that have been shown to mediate expression of cold-responsive genes of *Arabidopsis thaliana*. In this paper, we analyzed the *CbCOR15a* and *CbCOR15b* promoter sequence shown that there are two or one *cis-acting* elements in between -305 and -149, or -207 and -128, respectively. Deletion sequence of CbCOR15 promoter region were fused to the GUS reporter gene and introduced into *A. thaliana* plants. The analysis of independent transgenic lines using histochemical GUS staining method indicated that the *CbCOR15a* promoter sequences from -305 to -149, *CbCOR15b* promoter sequences from -207 to -128 is as necessary for gene expression of low temperature regulated. *CbCOR15* promoter displayed a slight activity in seedlings, mature rosette leaves, stem leaves, flowers and mature siliques, and after cold treatment, the promoter activity increased greatly in all tissues. *CbCOR15a* being more sensitive to cold than *CbCOR15b*. After cold treatment, the *AtCOR15* promoter activity was greatly induced in leaves, flowers and siliques, but not roots. In comparison with character of *AtCOR15* promoter indicated that *CbCOR15* being more sensitive to cold. The character may be related to strong cold acclimation ability of *C. bursa-pastoris*. © 2016 Friends Science Publishers

Keywords: *Capsella bursa-pastoris*; Cold Regulated (COR) gene; Promoter; Histochemical staining experiments

Introduction

Chilling stress can limiting plant growth, development and distributed. Based on plant tolerance, cold can be divided into freezing (<0°C) and chilling (0-15°C) temperatures (Zhou *et al.*, 2011). During the long of evolution history, plants have developed some adapt ability to resist low temperatures stress. These abilities known as cold acclimation contain increase the response of tolerance in low non-freezing temperatures (Thomashow, 1999) and alterations variety carbohydrate composition including in lipid, protein during cold acclimation (Steponkus and Lynch, 1989; Guy, 1990; Thomashow, 1990). Among these adaptive changes, playing a key structural role are the Cold Regulated (COR) and Dehydrin (DHN) proteins that function to stabilize membrane structure (Thomashow 1999; Koag *et al.*, 2009) or these protein changes may have important roles in the acclimation process (Thomashow,

1990). In *Arabidopsis thaliana*, five pairs COR genes have been found, including COR6.6 (Kurkela and Borg-Franck, 1992), COR15 (Lin and Thomashow, 1992; Wilhelm and Thomashow, 1993), COR47 (Gilmour *et al.*, 1992), COR78 (Horvath *et al.*, 1993) and COR413 (Hajela *et al.*, 1990). In each gene pairs have a high identity of nucleic acid sequence and are physically tandem linked array. In addition, the individual members of each gene pair showed some differences in gene regulation level. Among these proteins, COR15 was the first to be found and provides the first direct evidence for cold tolerance enhanced is related to the constitutive and continued expression of cold-induced genes. The COR15a and COR15b from *A. thaliana* is a chloroplast- targeted protein, its molecular weight is 15 kDa (Wilhelm and Thomashow, 1993). The COR15a and COR15b homologs gene are present in tandem on the chromosome 2, but their expression level to cold stresses response is differ temporally or spatially (Wilhelm and

Thomashow, 1993). Similarly, *COR15* two copies have been discovered in the *Brassica napus* species (Weretilnyk *et al.*, 1993), *Hordeum vulgare* (Cattivelli and Bartels, 1990; Crosatti *et al.*, 1996), *Triticum aestivum* (Shimamura *et al.*, 2006), *Chorispora bungeana* (Si *et al.*, 2009), *Cucurbita moschat* and *Citrullus lanatus* (Kang *et al.*, 2009), *B. oleracea* (Hadi *et al.*, 2011), *Capsella bursa pastoris* (Zhou *et al.*, 2012; Wu *et al.*, 2012). *COR15a* and *COR15b* protein are necessary for Arabidopsis to attain comprehensive freezing tolerance during cold acclimation; its function in *A. thaliana* seems to protect chloroplast membranes through binding and folding during freezing (Artus *et al.*, 1996; Thalhammer *et al.*, 2014). After exposing plants to low temperature, a number of *COR* genes transcript levels rapid increase dramatically and maintain elevated for as long as remain plants in the low temperature environments. In spite of, much study has shown that expression changes do indeed occur during cold acclimation in plants (Guy, 1990). But character of gene expression is different in various plants specific, such as, the *COR15a* gene promoter is inactive (or only low active) in many plants grown organs or tissues, exception anther, at 22°C temperatures using gene fusion experiments. When plant moved to cold temperature, these genes become activated in grown tissues and organs, exception in the roots (Baker *et al.*, 1994). In addition, the regulated patten of *COR15b* and *COR15a* are differential (Wilhelm and Thomashow, 1993). *BnCOR25* gene from *B. napus* was weakly expressed in leaves or roots and strong expressed in flowers, stems, hypocotyls and cotyledons using RT-PCR analysis. *BnCOR25* gene transcripts were highly accumulated in roots during cold treatment (Chen *et al.*, 2011).

In comparison with *A. thaliana*, *C. bursa-pastoris* possesses relatively strong tolerance ability to cold. However, cold tolerance ability to associate with the accumulation of *COR15* cold-induced transcript is not well understood. In *C. bursa-pastoris*, some *COR* genes, such as *CbCOR15a*, *CbCOR15b* have been characterized and are found to be low temperature induced gene (Wu *et al.*, 2012; Zhou *et al.*, 2012). In order to clarify the possible mechanisms involved in cold expression regulation of *COR* gene, which demonstrated the low temperature induced of *COR15a/b* gene requires the CRT/DRE/LTRE element. We investigated the induced and tissue specific expression of *COR15a/b* from *C. bursa-pastoris* using the promoter-reporter gene (*GUS*) fusions in transgenic *A. thaliana*. The results show that the CRT/DRE/LTRE element is a key element of *CbCOR15a* and *CbCOR15b* cold response. The results confirm that the *cis-acting* regulatory element is essential to adjust the expression of cold-regulated gene. Further, to investigate the different to transcript regulation of *COR15a/b* from *A. thaliana* or *C. bursa-pastoris*, we carried out some transgenic lines using *COR15a/b* promoter from *A. thaliana* or *C. bursa-pastoris* using b-glucuronidase (*GUS*) fusions, respectively. Under the cold treatments, the increase in *GUS* activity was found

in transgenic plant lines, indicating that cold induction of *COR15a* and *COR15b* from *C. bursa-pastoris* occurs at the transcriptional level.

Materials and Methods

Plants Growth and Treatments with Low Temperature

The generations of *C. bursa-pastoris* seeds was purchased from Shanghai Baiyulan Vegetable Seed Ltd. and are grown in the greenhouse. The conditions of greenhouse contain 16-h-light/8-h-dark cycle long photoperiod, 140 to 150 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ cool-white fluorescent illumination and 70% relative humidity at 22°C. Before germination, the seeds of *A. thaliana* (ecotype Columbia) were treated for 3 days at 4°C. The seeds were grown in growth chambers under at 100 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ constant light and 50% relative humidity. To perform low temperature treatments, *C. bursa-pastoris* and *A. thaliana* seedlings grown on MS solidified medium at 22°C, 2-week-old were transferred to a cold room (4°C; 8/16 h light/dark) and plants collected after 2d. Unless otherwise specified, three seeding constituted experimental unit.

Construction of the Plant Expression Vectors and Transformation of Plant

The total genomic DNA from *C. bursa-pastoris* and *A. thaliana* was isolated using the improve CTAB program (Murray and Thompson, 1980). The *Cbcor15a* or *Cbcor15b* promoter sequences were reported in our previous study reports (Wu *et al.*, 2012; Zhou *et al.*, 2012). The *AtCOR15a* or *AtCOR15b* promoter sequences from *A. thaliana* were reported in NCBI databases under gene number At2g42540 (*AtCOR15a*) or At2g42520 (*AtCOR15b*). To clone the *CbCOR15a*, *CbCOR15b*, *AtCOR15a*, *AtCOR15b* promoter sequence, four pair specific primers listed in Table 1 were used to amplify the four promoter sequence from *C. bursa-pastoris* or *A. thaliana* by PCR. To facilitate cloning into the vector pCAMBIA1301, a restriction enzyme site of *Pst*I was incorporated into a forward primer, whereas a *Bg*III site was flanked with a reverse primer. The PCR was performed using genomic deoxyribonucleic acid (DNA) isolated from young leaves of *C. bursa-pastoris* or *A. thaliana* as template under the following condition: The template was denatured 5 min at 94 °C followed by amplification 30 cycles, 1 min at 94°C, 30 s at 60°C, and 1 min at 72°C for program followed by 10 min at 72°C. After digestion with *Pst*I and *Bg*III, the CaMV35S promoter of pCAMBIA1301 (CAMBIA, Australia) be replaced by the amplified fragment. The plasmid was named pC**b**COR15aP::GUS, pC**b**COR15bP::GUS, pA**t**COR15aP::GUS or pA**t**COR15bP: GUS. Construction of vector model shown in Fig. 1. To clone the -459 to +97bp, -335bp to +97bp, -149 to +97bp fragments of *CbCOR15a* or -207 to +97bp, -128 to +97bp fragments from *CbCOR15b*,

five specific primers listed in Table 1 were used to amplify the five sequences by PCR. To facilitate cloning into the vector pCAMBIA1301, a restriction enzyme site of *Pst*I was incorporated into a forward primer. Construction diagram is shown in Fig. 1. *Agrobacterium* LBA4404 strain was transformed with these plant expression vectors by the freeze-thaw program and *A. thaliana* transformation by the floral dip program (Clough and Bent, 1998).

Molecular Analysis of Transgenic Plants

The transgenic T₀ seeds were selected on hygromycin (20 mg l⁻¹) and further were tested using PCR program to detect the specific fragment of the various constructs vector. After genomic DNA of leave was isolated, the gene-specific primers, hygromycin-specific primers or GUS-specific primers were used to amplify a specific fragment, hygromycin gene fragment GUS fragment, respectively, which demonstrated the presence of the *CbCOR15a* or *CbCOR15b* fragments, the hygromycin gene or GUS gene. These primers were listed in Table 1.

RT-PCR Method

Total RNA was isolated from leave tissues of 200 mg, treated with the deoxyribonuclease I to remove any DNA contamination, was reversely transcribed using Plant RNA Mini Kit (Watson Biotechnologies, Inc, China), DNase I Kit (Promega, Madison, WI, USA), or PrimeScript[®] RT Master Mix Perfect Real Time Kit (TaKaRa, China), respectively. For PCR amplifications, first cDNA strand was used as template using specific GUS primers described in Table 1. The *A. thaliana* tublin gene (Genbank number: M17189) was used as the control.

Histochemical Staining

GUS staining program was carried out as described by Jefferson *et al.* (1987). Briefly, various tissues were incubated in staining solution and vacuum infiltrated for 15 min. The staining buffer containing 7.2 mM ethylene diamine tetraacetic acid (EDTA), 3 mM potassium ferrocyanide, 42.3 mM sodium phosphate, 57.7 mM disodium phosphate, 0.005% Triton X-100 and 0.075% 5-bromo-4-chloro-3-indolyl-β-D- glucuronic acid (X-Gluc). Afterward the tube were put in incubator overnight at 37°C and de-stained using 75% ethanol for five times at 60°C until their chlorophyll were clear. The GUS activity was observed using microscope (Zeiss Scope A1, Zeiss, Germany).

Statistical Analysis

The effects of the tissue type and treatments were analyzed with the analysis of variance (ANOVA). P values < 0.05 were considered significant.

Result

Sequence Analysis of CbCOR15a and CbCOR15b Promoters

The gene expression is driven by its promoter. There are some regulatory elements in their promoter's sequences that control these gene expressions. Several genes to the freezing- or cold-induced responsive contain one or more *cis-element* in their promoter region (Wang and Hua, 2009). These element contain C-repeat element (CRT), DRE (dehydration responsive element) or LTRE (low temperature responsive element) (Baker *et al.*, 1994). The CCGAC sequence, conserved core sequence is the binding region to the cold specific CBFs/DREBs transcriptional activators (Stockinger *et al.*, 1997) that enhance the expression level of cold responsive COR genes which subsequently increase plants cold resistance (Mantas *et al.*, 2010). To examined whether *CbCOR15a* and *CbCOR15b* gene is induced by low temperature through the CRT/DRE/LTRE element in the promoter, we analysis the *cis-element* by the PLANTCARE database (<http://bioinformatics.psb.ugent.be/webtools/plantcare/html/>). These results shown *CbCOR15a* and *CbCOR15b* promoter sequences contain two or one CRT/DRE/LTRE elements, respectively. We analyze a series of transgenic positive plants to test whether this CRT/DRE/LTRE element is also involved in low temperature induction of *CbCOR15a* and *CbCOR15b*. Lines **CbCOR15aP**, **CbCOR15aP3**, **CbCOR15aP2** and **CbCOR15aP1** contain -939/+97, -459/+97, -335/+97 and -149/+97 of the *CbCOR15a* promoter sequences from transcription initiation site, respectively. Lines **CbCOR15bP**, **CbCOR15bP2** and **CbCOR15bP1** contain -1037/+97, -207/+97 and -128/+97 of the *CbCOR15b* promoter sequences from transcription initiation site, respectively (Fig. 2). These plants were grown on plates for about 14 days at 22°C, and then seedlings were shifted to 4°C. To GUS activity sufficiently detection, we stain the tissue in a longer induction time (2 days). There was no GUS activity in any of these lines grown at 22°C. However, **CbCOR15aP**, **CbCOR15aP2**, **CbCOR15aP3** and **CbCOR15bP** and **CbCOR15bP2**, but not **CbCOR15aP1** and **CbCOR15bP1**, showed GUS activities at 4°C (Fig. 3). The sequence of **CbCOR15aP** and **CbCOR15aP3** contain two CRT/DRE/LTRE elements, the sequence of **CbCOR15bP** and **CbCOR15bP2** contain one CRT/DRE/LTRE element, respectively. **CbCOR15aP1** and **CbCOR15bP1** do not contain any CRT/DRE/LTRE element, so in all organizations are not observed in GUS staining. **CbCOR15aP2**, **CbCOR15aP3** contain one CRT/DRE/LTRE element, both are not active at room temperature, but active were induced by low temperature. **CbCOR15aP2** and **CbCOR15aP3** was no significant difference in low temperature activity. **CbCOR15aP** contain two CRT/DRE/LTRE element, are low active at room

Table 1: Primers used in this work

Primers	Sequence(5'---3')
CbaP-Pst	TActgcagGTGAACATGGGGAGAGTGTA
CbaP-Bgl	CGagatctACCATCGCCATGAGTTATCTCTTTTAAAGTTTTTTGGGTGCA
CbbP-Pst	CGtgcagGATCAGTCTGTGTAATGA
CbbP-Bgl	CGagatctACCATCGCCATGAGAGATATG
Atap-Sac	CGTGCAACTTTTCAGAGCTCGC
Atap-Nco	CCCATGGGAGAGAGATCTTTAAGATGTGA
Atbp-Sac	GTTTAAAGTGCTTGAAATTGAGCTCGC
Atbp-Nco	CCCATGGGAGGACTCTTAAAGATGTT
CbaP1-Sac	GCgagctcCACGTGGCCAAAA
CbaP2-Sac	GCgagctcCACGTGAAGAGAATGAG
CbaP3-Sac	GCgagctcAITTACGGACCAATGTT
CbbP1-Sac	GCgagctcAACGAACAAAACTCTT
CbbP2-Sac	GCgagctcTGGCCGACCTCTTTT
GUS-F	GCTTACACCACGCCGAACACCTG
GUS-R	TCTTCAGCGTAAGGGTAATGCGAGGTA
tublin-F	ATGCGTGAGATTCTTCACATCC
tublin-R	TGGGTACTCTTCACGGATCTTAG

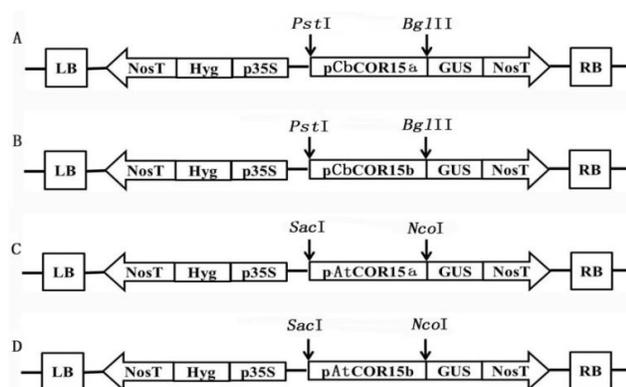


Fig. 1: The construction model of plant expression vectors
 A: Construction model of CbaP::GUS, B: Construction model of CbbP::GUS, C: Construction model of Atap::GUS, D: Construction model of Atbp::GUS

temperature, but active were induced by low temperature. The active CbCOR15aP was high than CbCOR15aP2 and CbCOR15aP3 in low temperature. These results show that the CRT/DRE/LTRE element is a key element of *CbCOR15a* and *CbCOR15b* cold response.

Comparing Analysis of *COR15* Promoters from *C. bursa-pastoris* and *A. thaliana*

Previous experiments about histochemical staining indicated that the *COR15a* promoter at normal temperature can be stained deeply blue only in anther of plants, in contrast, in the leaves, at cold-treated can be stained deeply blue in stem, leaf and apical meristem. However, the roots and mature ovules of these seedlings was detected no blue (Baker et al., 1994). The nucleic acid sequence of *COR15b* has a high degree identity to *COR15a*, but differences in regulation (Wilhelm and Thomashow, 1993). In the study, four expression vectors, including *CbCOR15aP::GUS*, *CbCOR15bP::GUS*, *AtCOR15aP::GUS* and *AtCOR15bP::GUS*, were

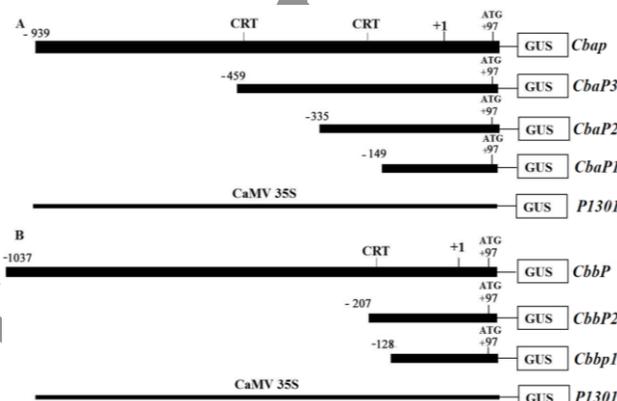


Fig. 2: Regions of *cor15* included in the *cor15a-gus* fusions.
 A. The *cor15a* sequences that were fused to *gus* are indicated. B. The *cor15b* sequences that were fused to *gus* are indicated. The numbers refer to the nucleotide sequence. The locations of the CRT, transcription initiation site and translation initiation site (AUG) are shown

constructed and transferred into *A. thaliana* in order to analyze the promoter tissue-specific expression activity in normal temperature or cold treatment. Seeds of T₂ lines (transgenic *CbCOR15aP*, *CbCOR15bP*, *AtCOR15aP* and *AtCOR15bP* positive Arabidopsis plants) were plants in MS₀ solid medium. For the histochemical GUS staining assay, the 6-week-old seedlings were collected at 22°C and 4°C treatment for 2 d, respectively. The results showed that *CbCOR15a* promoter displayed a slight activity in 22°C in seedlings, mature rosette leaves, stem leaves, flowers and mature siliques, and after 4°C cold treatment, the activity increased greatly in all tissues. *CbCOR15b* promoter was detected inactive in 22°C, but after 4°C cold treatment, the activity was greatly induced in leaves, flowers, siliques and roots. The active of *AtCOR15b* promoters are same to *AtCOR15a*, after 4°C cold treatment, the promoter activity was no induced in roots (Fig. 3). This result is consistent

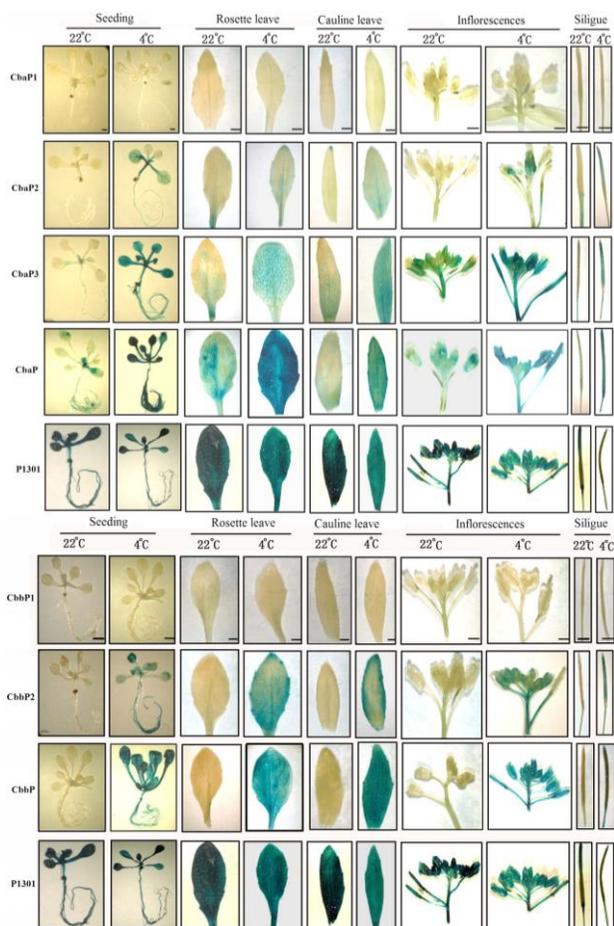


Fig. 3: Tissue-specific expression of the various *gus* chimeric gene in control and cold-treated plants. A. Plants transformed with the -930/+97 (CbaP), -459/+97 (CbaP3), -335/+97 (CbaP2) and -149/+97 (CbaP1) *cor15a-gus* chimeric gene were grown under different conditions and subjected to histochemical staining to localize GUS activity. B. Plants transformed with the -1037/+97 (CbbP), -207/+97 (CbbP2) and -128/+97 (CbbP1) *cor15b-gus* chimeric gene were grown under different conditions and subjected to histochemical staining to localize GUS activity. Transgenic plants grown for 21 days at 22 °C then cold-treated at 4°C for 1 day. Transgenic plants carrying the CaMV 35S-*gus* fusion (CaMV 35S) as control. The scale bar represents 2 mm

with RT-PCR analysis (Zhou *et al.*, 2012). At the same time the transcript levels of the GUS genes were determined in transgenic plants using low temperature treated and control by RT-PCR method, the result is shown in Fig. 4. The results showed the GUS fusions containing *Atcor15aP*, *Atcor15bP*, *Cbcor15aP* and *Cbcor15bP* were strongly responsive to cold. To cold-treated plants, GUS transcripts accumulated to higher levels than the control plants. In contrast, the level of GUS expression is higher containing *Cbcor15aP* or *Cbcor15bP* than containing *Atcor15aP* or

Atcor15bP (Fig. 4). The result shown that the *COR15* genes differ in their cold sensitivity: *COR15* from *C. bursa-pastoris* being more sensitive to cold than *COR15* from *A. thaliana*; *COR15b* from *C. bursa-pastoris* is being more sensitive to cold than *COR15a*.

Discussion

The COR gene has been described from many plants, little is known about the gene expression pattern from various species and its multiple members. The promoters of many COR genes such as *COR15a/b* (Wilhelm and Thomashow, 1993; Baker *et al.*, 1994), *RAB18* (Lång and Palva, 1992), *COR78* (Yamaguchi-Shinozaki and Shinozaki, 1993), *KINI2* (Kurkela and Franck, 1990; Kurkela and Borg-Franck, 1992) from *A. thaliana* contain highly conserved *cis-elements* such as CRT, DRE or LTRE element (Stockinger *et al.*, 1997), and consequently, expressions of COR genes are mediated by cold. Under cold stress, the upstream regulators named inducers of CBF expression (ICE) act as a positive regulator of CBFs, while CBFs regulate the cold responsive (COR) genes by binding to the CRT/DRE element (Lissarre *et al.*, 2010). Our promoter-fusion experiments found that *CbCOR15a* contain two *cis-acting* CRT/DRE elements between nucleotides -939 and +97 and *CbCOR15b* contain one *cis-acting* CRT/DRE element between nucleotides -1037 and +97 that can drive strong cold regulated gene expression. This found confirm that *CbCOR15* genes contain a cold-regulatory element in their promoter. Previously study shown expression in almost all plants tissues about *AtCOR78-gus* (Horvath *et al.*, 1993) and *AtRD29a-gus* gene (Yamaguchi-Shinozaki and Shinozaki, 1993) was either very low or undetectable in non-acclimated condition. However, relatively high expression in the *AtCOR15a-gus* can be detected in the anther of control plants. Ours result shown relatively high expression levels of the *CbCOR15a-gus* can be detected in the anthers in 22°C. The molecular basis for lack of expression of the COR promoter in some tissues at normal growth temperature is unclear. In addition, these found indicate that the expression level of various COR gene is up-regulated by cold to most plant tissues, but not whole. Such as, after 4°C cold treatment, the activity of *AtCOR15a* promoter was greatly induced in leaves, flowers and siliques, but not roots (Baker *et al.*, 1994). But, the activity of *AtCOR78* promoter was greatly induced in the flower sepals, leaves, stems, roots of plants by cold-treated. However, these plants tissue, such as stigmas, anthers, styles of flowers or ovaries no activity was detected (Horvath *et al.*, 1993). Ours study shown the activity of *CbCOR15* promoter can be induced greatly in all tissues, includes seedlings, rosette leaves, cauline leaves, inflorescence and siliques. The results indicate that different *cis-acting* elements are responsible for the different expression patterns under conditions of normal or low temperature.

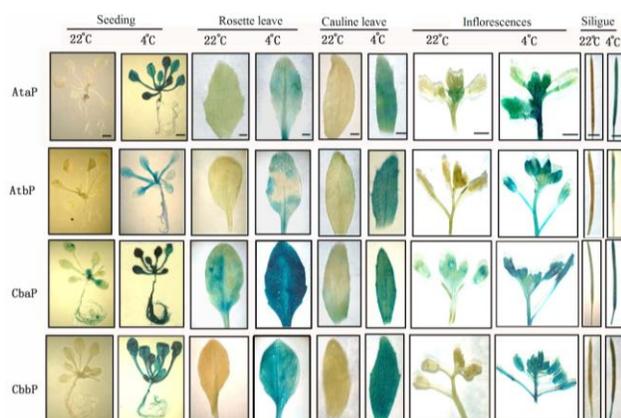


Fig. 4: Tissue-specific expression of the four *gus* chimeric gene in control and cold-treated plants. Plants transformed with the -930/+97 (CbaP), -1037/+97 (CbbP), -335/+97 (AtaP) and -149/+97 (AtbP) *cor15-gus* chimeric gene were grown under different conditions and subjected to histochemical staining to localize GUS activity. Transgenic plants grown for 21 days at 22°C then cold-treated at 4°C for 2 day. Transgenic plants carrying the CaMV 35S-*gus* fusion (CaMV 35S) as control. The scale bar represents 2 mm

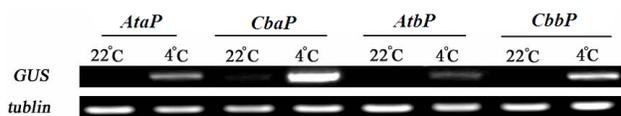


Fig. 5: Expression of *cor15-gus* chimeric genes in response to low temperature using RT-PCR analysis. Transgenic plants carrying the indicated *cor15-gus* fusions (see Fig. 3) were grown in pots at control temperature (22°C) and then treated at low temperature (4°C) for 1 day. Total RNA was isolated from the transgenic *Arabidopsis* leaves

The main function of COR15a protein in plant is stabilizing thylakoid membranes against freezing-induced damage, and as a result, constitutive overexpression of the COR15 gene resulted in a significant increase in cell freezing-tolerance of plant (Artus *et al.*, 1996). It was indicated that the accumulation of COR proteins is related with plant frost resistance. Under field conditions, plant accumulated more COR proteins in winter than spring (Grossi *et al.*, 1998). So COR15 are necessary for plant to attain full freezing tolerance during cold acclimation. Ours study shown the activity of *CbCOR15* can was induced greatly in all tissues by cold, the *CbCOR15* gene plays a positive role in conferring freezing/cold tolerance, suggesting that it may function in *C. bursa-pastoris* response and tolerance to cold stress. Ours findings for increasing the freezing tolerance of crop species have potential application using transgenic technology. It is favorable to drive the of those genes expression by use plant endogenous stress-inducible promoter instead of the 35S

promoter to prevent the 35S promoter leading to side effect, such as dwarfism or delay flowering. The cold inductive activities of *CbCOR15a/b* promoter provide a potential element in improvement of cold resistance crops using transgenic technology.

Conclusion

The ability of plants to adapt to cold is associated to the expression of COR gene. The *cis-acting* element is necessary for cold-regulated gene expression. We results showed that the 5' region of *CbCOR15a* and *CbCOR15b* contains two and one *cis-acting* element, respectively. The *CbCOR15* promoter displayed a slight activity in seedlings, mature rosette leaves, stem leaves, flowers and mature siliques, and after cold treatment, the promoter activity increased greatly in all tissues. But after cold treatment, the *AtCOR15* promoter activity was greatly induced in leaves, flowers and siliques, but not roots. The *C. bursa-pastoris COR15* is more sensitive to cold than *A. thaliana COR15* and *C. bursa-pastoris COR15b* is more sensitive to cold than *C. bursa-pastoris COR15a*. The character may be related to strong cold acclimation ability of *C. bursa-pastoris*.

Acknowledgements

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